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THE INFLUENCE OF PIROXICAM, A NON-SELECTIVE CYCLOOXYGENASE INHIBITOR, ON AUTONOMIC NERVOUS SYSTEM ACTIVITY IN EXPERIMENTAL CYCLOPHOSPHAMIDE-INDUCED HEMORRHAGIC CYSTITIS AND BLADDER OUTLET OBSTRUCTION IN RATS

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Abstract: Signs and symptoms of secondary overactive bladder (OAB) are observed both in course of infravesical obstruction of urine outflow in patients with benign prostatic hyperplasia, and as a result of development of hemorrhagic cystitis (HC) following administration of cyclophosphamide (CP). Non-steroidal anti-inflammatory drugs (NSAIDs) alleviate symptoms of bladder overactivity reducing local synthesis of prostaglandins (PGs), but precise effects of those agents on functions of the autonomic nervous system (ANS) in course of OAB remain unknown. The purpose of this study was to evaluate the effect of piroxicam-induced prostaglandins (PGs) synthesis block on activity of the ANS in two experimental models of secondary OAB: bladder outlet obstruction (BOO) and cyclophosphamide-induced HC (CP-HC), by heart rate variability analysis (HRV). The experiment was performed on a group of rats with surgically induced 2-week BOO, and on a group of rats that were administered CP five times, with corresponding control groups. Study animals were given piroxicam (PRX) *i.p.* in two doses: 2 and 10 mg/kg b.w.
In the BOO model, PRX in both doses revealed a trend for reduction of value of all non-normalized components of HRV. The lower PRX dose caused an increased nHF value, and PRX administered in the dose of 10 mg/kg b.w. caused an increase of the nLF value. In the CP-HC model, the lower PRX dose caused a trend for an increase of values of all non-normalized components, and the higher dose – for their decrease. Both doses of PRX in that model caused increase of the nLF value. Inhibition of PGs synthesis caused changes of ANS function in both models of OAB. Both in BOO and in CP-HC, PGs seem to be ANS-activating factors, responsible for maintenance of a high parasympathetic activity. In both models, inhibition of PGs synthesis with PRX administered at the dose of 10 mg/kg b.w. lead to functional reconstruction of ANS, with marked sympathetic predominance. That may contribute to reduction of the bladder contractile action and improvement of its compliance in the filling period, which was demonstrated by other authors in urodynamic tests for NSAIDs.

Keywords: overactive bladder, cyclophosphamide, bladder outlet obstruction, prostaglandins, autonomic nervous system, heart rate variability

Abbreviations: ANS – autonomic nervous system, BOO – bladder outlet obstruction, BPH – benign prostatic hyperplasia, BWW – bladder wet weight, COX – cyclooxygenase, CP – cyclophosphamide, CP-HC – cyclophosphamide-induced hemorrhagic cystitis, HF – high frequency (HRV spectral, non-normalized component), HRV – heart rate variability, LF – low frequency (HRV spectral, non-normalized component), nHF – normalized high hrequency (HRV spectral, normalized component), nLF – normalized low frequency (HRV spectral, normalized component), NSAIDs – non steroidal antiinflammatory drugs, OAB – overactive bladder, PGs – prostaglandins, PRX – piroxicam, rMSSD – the square root of the mean squared difference of successive normal-normal (R-R) intervals, R-R – an interval between two subsequent R waves in ECG recording, SDNN – standard deviation of all normal-normal (R-R) beats, TP – total power (HRV spectral, non-normalized component), VLF – very low frequency (HRV spectral, non-normalized component)

Symptoms of the urinary bladder overactivity, with an impairment of the filling phase and its reduced compliance, are common problems of the lower urinary tract. According to the currently applied terminology, disorders suggesting some dysfunctions of the bladder filling phase (urinary urgency) and micturition (polyuria, frequent passage of small amounts of urine, urine incontinence), constitute a basis for diagnosis of the overactive bladder syndrome (OAB) (1, 2).

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The disease is an idiopathic disorder, or is a result of action of various etiological factors leading to detrusor overcontractility with reduced filling phase of the bladder. A general pathophysiological description of idiopathic OAB assumes, that its development is a result of complex disorders, both of myogenic (abnormal electric coupling of smooth muscle cells of the vesicular wall, contributing to contractile overreactivity) and neurogenic character. A precise pathophysiological description of idiopathic OAB exceeds the framework of this paper, and all necessary information may be found in numerous reviews in that area (3–5), also published by us (6). Bladder overactivity is also observed in course of various organic disorders of the lower urinary tract. Symptoms of OAB occur in patients treated with cytostatic alkylating drugs belonging to the group of oxazaphosphorins (e.g., cyclophosphamide), accompanying symptoms of hemorrhagic cystitis (HC; cyclophosphamide-induced HC; CP-HC). The pathogenesis of hemorrhagic cystitis is associated with development of the bladder inflammatory condition – that, according to the OAB neurogenic theory, is co-responsible for changing the activity of afferent fibres and, at the same time, the efferent control of the bladder function. The key pathogenetic factor is release of acrolein (as a result of cyclophosphamide metabolism in the bladder), as a factor initiating some complex immunological-inflammatory changes, with overproduction of NF-κB, TNF-α and overactivity of cyclooxygenases (COX) (7, 8). The pathophysiological description of that disorder has been also published in one of our earlier papers (9). It is also generally known that patients with benign prostatic hyperplasia (BPH) also present symptoms of bladder overactivity. Pathogenesis of bladder overactivity in BPH patients depends on the bladder reconstruction secondary to the existing sub-vesicular block and urine outlet obstruction (10).

The above mentioned disorders may be relatively easily studied on animal models. According to the literature, it is possible to cause a hemorrhagic cystitis by four intraperitoneal administration of 75 mg/kg b.w. cyclophosphamide to rats (the CP-HC model) (11, 12). On the other hand, BPH may be experimentally reflected by surgical creation of a partial bladder outlet obstruction (the BOO model) obtained by partial ligation of the proximal section of the urethra (13, 14).

In both above mentioned cases of secondary OAB, disturbances of the autonomic bladder control are an additional pathogenetic element. In our previous studies, we have demonstrated that in experimental CP-HC, the autonomic nervous system (ANS) activity became reduced with a proportional reduction of both sympathetic and parasympathetic tonus (15). However, evaluating function of the ANS in a 2-week BOO model in a rat, we have also found features of reduction of global autonomic activity, but with a reduced parasympathetic tonus and a relative sympathetic dominance (16). Autonomic abnormalities observed in the above mentioned experimental models may depend on altered paracrine function of the urothelium, being a result of the existing inflammatory condition (CP-HC) or excessive muscularization of the bladder due to its overpressurising. Those disorders cause release of prostaglandins (PGs) inside the bladder. PGs may be co-responsible for excessive sensitization of the bladder sensory fibres and reflexive change of tonus of efferent fibres.

It is generally known that PGs are synthesized *de novo* from arachidonic acid, in a multi-stage process with participation of cyclooxygenase (currently identified with prostaglandin H synthase, or prostaglandin endoperoxidase) and individual cellular synthases for given prostanoids. Cyclooxygenase is a molecular binding point for non-steroidal anti-inflammatory drugs (NSAIDs), that exert an analgesic and antipyretic effect by inhibition of synthesis of prostaglandins. There are several isoforms of cyclooxygenases (COX1 – constitutive, COX2 – inflammatory-induced, COX3 – central form), constituting a basis for pharmacodynamic classification of NSAIDs, depending on their – selective or non-selective – effect on individual COX isoforms (17, 18).

Studies demonstrated that PGs are synthesized locally in the bladder, both in the urothelium and the muscular coat, indicating a complex, endocrine control of the bladder function (19, 20). Intravesical PGs administration to rats caused reduction of the bladder compliance and its overreactivity (21, 22). Experimental studies demonstrated as well an increased COX expression, both in the BOO model (23) in response to the bladder overpressure and its dilation, and in the model of post-cyclophosphamide bladder injury, as a result of internal inflammation (24). Some recent studies demonstrated also that interstitial cells of Cajal, being an electric "pacemaker" of the bladder, controlling its motor function (similarly to the digestive tract) also present a high COX expression (both isoforms 1 and 2) (25).

Considering pleiotropic character of prostanoids, determination of their potential role in the bladder overactivity in course of post-cyclophosphamide, hemorrhagic cystitis and benign prostatic

hyperplasia, in context of their effect on autonomic regulation, seems important. Therefore, the purpose of this study was to determine an indirect – through a pharmacological inhibition of prostaglandin synthesis with piroxicam (PRX), a non-selective COX inhibitor – effect of prostaglandins on activity of the autonomic nervous system in experimental models of post-cyclophosphamide, hemorrhagic cystitis (the CP-HC model) and of a partial bladder outlet obstruction (the BOO model). Currently, a non-invasive evaluation of the autonomic nervous system – both for clinical and experimental purposes – is allowed by examination of the heart rate variability (HRV). The HRV analysis is based on evaluation of variability of R-R intervals in the ECG record. Those intervals fluctuate constantly due to the fact that the heart remains under constant autonomic control. That parameter is a starting point for time-domain HRV analysis, yielding some derivative parameters. It also allows determination of a HRV spectrum with its basic components (HRV spectral analysis) (26, 27). Therefore, evaluating an autonomic control of the heart, some indirect conclusions of the ANS functional status may be drawn.

EXPERIMENTAL

The study was carried out following a consent of the 1st Local Bioethics Committee in Kraków (No. 126/2010 and 28/2013).

Animals

Sixty 6-weeks-old Wistar rats obtained from the central animal house of the Pharmaceutical Faculty of the Jagiellonian University Collegium Medicum in Kraków were used for experiments. For acclimatization to new living conditions, for the first week animals were placed in five collective cages, with unlimited access to a standard laboratory feed (Labofeed, Kcynia) and water. Constant temperature of 22°C was maintained in the room. At the beginning of the experiment, rats were randomized into study groups, 10 animals in each group. During the experiment, animals in the particular group lived together in the same cage (10 animals per cage), and had unlimited access to water and feed.

General outline of the experiment

The experiment was run on animals with BOO, treated (BOO + PRX) and untreated (control) with piroxicam. Also animals with post-cyclophosphamide hemorrhagic cystitis were divided into two groups: treated with piroxicam (CP-HC + PRX) and untreated (control). Moreover, both in the BOO + PRX, and CP-HC + PRX group we have used two doses of PRX: 2 mg/kg b.w. and 10 mg/kg b.w. (choice of doses was based on previously published experimental studies using PRX (28, 29)). Therefore, finally six study groups were formed: BOO (control of the BOO model; group 1); BOO + PRX 2 mg/kg b.w (group 2); BOO + PRX 10 mg/kg b.w. (group 3); and CP-HC (control of the CP-HC model; group 4); CP-HC + PRX 2 mg/kg b.w. (group 5); CP-HC+PRX 10 mg/kg b.w. (group 6).

CP-HC model

In thirty animals, an experimental model of hemorrhagic cystitis with its overactivity was created by four (every two days – on the day one, three, five and seven) intraperitoneal administrations of cyclophosphamide – CP (Sigma Aldrich) – at the dose of 75 mg/kg b.w. The CP solution was prepared each time *ex tempore* before administration. According to the literature, dosage scheme leads to hemorrhagic cystitis following the fourth dose (11, 12). Ten animals received CP only, and formed a control group (group 4), and other 20 rats received additionally piroxicam (Feldene, Pfizer, ampoules 20 mg/mL) in two doses: 2 and 10 mg/kg b.w., as described above. PRX, the same as CP, was administered intraperitoneally four times, two hours following the administration of CP. In the group of animals receiving 10 mg/kg b.w. PRX, four animals died – therefore, only six animals were considered in the final analysis. In the CP-HC + PRX 2 mg/kg b.w. group one animal died, therefore HRV records were finally obtained from 9 animals. The controls received normal saline in volume corresponding to the volume of PRX instead of PRX. All animals in that group survived administration of the fourth dose of CP, but a majority of them were in overall poor condition. We have even observed a body weight reduction in those animals.

BOO model

Another 30 animals had the proximal section of urethra partially surgically ligated, in order to create the bladder outlet obstruction. According to the literature, a condition clinically corresponding to BPH with bladder overactivity develops within two weeks post the surgery (13, 14). The procedure was conducted under pentobarbital anesthesia (Morbital, Puławy, 40 mg/kg b.w. administered *i.p.*). Following the anesthesia, a medial cut was made in the projection over the bladder. Exposed bladder was carefully separated from the surrounding fatty tissue, and the urethra was catheterized (polyethylene catheter, diameter 0.58 mm), ligating the proxi-

mal section of the urethra around the catheter. After the catheter was removed, abdominal integument and skin were sutured in layers with standard surgical sutures (Medilen 4/0 USP; cutting needle DS2, 3/8). The surgical wound was sprayed with neomycin and the skin was sprayed with oxycort to minimize the risk of post-surgical infection. Two first days after the surgery were treated as a convalescence period. On the day three, PRX was administered *i.p.* at the dose of 2 or 10 mg/kg b.w. in both BOO + PRX subgroups (groups 2 and 3), or normal saline was administered at the volume corresponding to the volume of PRX in the control BOO group (group 1). PRX or normal saline were administered, similarly to CP-HC + PRX/CP-HC groups, four times – on the day three, seven, eleven and fifteen after the surgery. Two animals in the control BOO group did not survive the procedure. Therefore, that group consisted finally of 8 animals. Moreover, 2 animals treated with PRX at the dose of 2 mg/kg b.w. and 3 animals treated with PRX at the dose of 10 mg/kg b.w. died before reception of the final dose. Therefore, HRV records were obtained from 8 animals in the BOO + PRX 2 mg/kg b.w. group and from 7 animals in the BOO + PRX 10 mg/kg b.w. group.

Body weight and excreted urine volume measurement

On the first day of the experiment and on the last day (the day of HRV records registration) body weight of animals was measured in all study groups. Moreover, on the first day of the experiment, we measured a daily urine output in healthy animals randomized to control groups in BOO and CP-HC models (groups 1 and 4) and in animals treated with PRX (groups 2, 3, 5, 6) – after the last PRX dose and on the day preceding the HRV registration.

HRV records

In groups 1–3 HRV was recorded on the day fifteen (day after the last dose of PRX administered to animals in groups 2 and 3 and normal saline to animals in the group 1). In groups 4–6 HRV was recorded on the day eight (after the last dose of CP and PRX or normal saline). For all rats the HRV registration was performed under urethane anesthesia (Sigma Aldrich; 1200 mg/kg b.w.), considering the literature reports of the relatively lowest effect of urethane on the autonomic nervous system, compared to other anesthetics (30, 31). After an animal was anesthetized, shaved and placement of ECG gel, three disposable electrodes Ag/AgCl (E30, Sorimed, Poland) were placed in standard places. Two of them were active electrodes and one a reference, in order to obtain a single-channel, bipolar record. During the record, animals were placed under a heating lamp to prevent chilling that could negatively affect the ANS activity. ECG was registered at rest for 20 min (Polygram, ADInstruments). Following registration, records were analyzed for time- and spectral (frequency) HRV analysis, with calculation of standard parameters, according to generally accepted guidelines (26, 27). The time analysis involved: mean RR [ms] – a mean duration between subsequent R-R waves, maximum and minimum duration between subsequent R-R waves [ms], range of variability of R-R intervals [ms], mean heart rate [bpm], SDRR – standard deviation of all R-R intervals, and rMSSD – square root of mean sum of differences between R-R waves.

Evaluated in the spectral analysis were: total power (TP), powers of individual non-normalized components of the spectrum in the range of very low frequency (VLF), low frequency (LF), and high frequency (HF); all expressed in power units [ms^2], LF/HF ratio and values of normalized parameters nLF and nHF (expressed in normalized units [n.u.]). The following ranges were accepted for individual components of the spectrum: $0.18 < VLF < 0.28 < LF < 0.78 < HF < 3$.

Collection of bladders

Following HRV recording, animals were sacrificed with a lethal dose of pentobarbital (Morbital, Puławy, Poland; 100 mg/kg b.w.) for weight and histopathological analysis of their bladders. The bladder was collected from each of the study animals, following a previous separation from the surrounding fatty tissue and voiding. According to the literature data, measurement of the bladder wet weight (BWW) may be treated as an indirect evidence of the bladder reconstruction induced by inflammation (in the CP-HC model) or by outlet obstruction (in the BOO model), associated with its overactivity (32–34). Directly after collection, bladders were weighed on an analytic scale and then placed in 4% formalin solution with PBS for further histopathological evaluation. During the histopathological procedure, the urinary bladders collected during autopsy were rinsed in the saline solution. Next, they were strengthened for 24 h in the 8% formalin with phosphate buffer solution (PBS; pH 7.4). Afterwards, the samples were rinsed in the slow running water for 2 h, and then they were drained in the successive growing concentration (50–100%) of ethanol solutions. Before sinking into paraffin, the samples were moved through both pure xylene solu-

tion and mixture of xylene and paraffin (1 : 1) and incubated for 2 h with the maintenance of 37°C. Next, the individual tissue fragments were moved twice to clean paraffin and incubated again in temperature 62°C. In the end, after 2 h, the samples were sunk into blocks and after hardening were cut using the microtome. The obtained scraps were placed on the microscopic slides and dried in the thermostat at 37°C. The finally prepared microscopic sections were stained with hematoxylin eosin method (HE) to enable the histologic evaluation of the inflammatory process intensification.

Statistical analysis

Obtained results were analyzed separately for groups 1–3 and 4–6 using the Bartlett test, at $\alpha = 0.05$. Then, paired results were analyzed for groups: 1–2, 1–3 and 4–5, 4–6 using the Fischer Snedecor test at the same α value. Calculated HRV values, considering lack of their normal distribution, were converted into logarithmic values (natural logarithms) for the sake of the statistical analysis.

RESULTS

Body weight and urine volume measurements

At the beginning of the experiment, body weight of study animals was 188.30 ± 11.83 g. In the BOO population (group 1), on the day 15 of the experiment, a mean body weight of study animals was 203.20 ± 8.58 g, and in the CP-HC model (group 4), on the day 8 of the study, a day after the final dose of CP, a mean body weight was reduced to 181.67 ± 10.62 g, which was associated with poor general condition of animals in that group.

Before the experiment, healthy rats excreted a mean volume of 9.50 ± 4.37 mL of urine per day. In the BOO model (group 1), on the day 14 following induction of BOO we recorded a mean daily urine output of 6.90 ± 1.03 mL, and in the CP-HC population (group 4), on the day seven following the last dose of CP – 14.83 ± 6.43 mL. Measurements of daily urine output were intended as an additional, indirect evidence of the bladder function disorders in examined models. We did not analyze any precise changes of those parameters under influence of PRX.

Evaluation of bladder wet weight of collected bladders

In the BOO group (group 1), on the day 15 of the experiment, the bladder wet weight achieved 0.26 ± 0.24 g, and in the CP-HC population (group 4), on the day 7 following the last dose of CP – 0.17 ± 0.04 g. Similarly to the measurement of daily urine output and according to the above mentioned literature (32–34), the BWW measurement was intended as an indirect evidence of the bladder function disorders in particular studied models. The parameter wasn't also analyzed for effect of PRX. Therefore, we gave up analogous measurements in PRX-treated groups.

Conclusions of histological analysis of collected bladders

According to the pathomorphological evaluation, bladders collected from animals with experimental bladder outlet obstruction (control group of the BOO model; group 1) demonstrated signs of edema and congestion of the bladder wall, with a minimal hyperplasia of the muscular layer. In the group 4 (control group of the CP-HC model) a clear edema and signs of congestion (mostly of the cystic mucosa) were found, and also signs of focal proliferation of fibroblasts in the mucosal lamina propria, mostly around some fine, submucosal blood extravasations. Fine lymphocytic inflammatory infiltrations were visible in vicinity of vessels of the mucosal lamina propria. Epithelium of the bladder lining demonstrated focal ulceration with signs of clear proliferation of cells and of anisonuleosis focalis et papillosis. The bladder wall muscular coat was normotypic.

HRV analysis in the BOO model

Time-domain analysis

Parameters of the HRV time analysis were not significantly different in both groups treated with PRX and in the control group 1. The population treated with 2 mg/kg b.w. PRX presented the lowest value of the mean R-R interval, with the highest heart rate. Animals treated with the higher dose of PRX (group 3) demonstrated the highest SDNN value. Results of the HRV time-domain analysis of animals in the BOO model are presented in Table 1.

Spectral-domain analysis

Spectral analysis of the BOO model revealed a trend for reduction of the total power of the spectrum and its non-normalized components in both groups treated with PRX compared to the control group. Significant differences were observed for normalized spectral parameters and their mutual relations – in the group 2 (lower PRX dose) a clear dominance of nLF was observed with reduction of the nHF value; in the group 3 (higher PRX dose) that correlation was precisely reverse.

Results of the HRV spectral analysis of animals in the BOO model are presented in Table 2.

Table 1. Time-domain HRV analysis results in rats with experimental bladder outlet obstruction (BOO) model treated with piroxicam (PRX).

HRV time-domain parameter	Studied groups			Statistical analysis (for ln values)	
	group 1 BOO control	group 2 BOO + PRX 2 mg/kg b.w.	group 3 BOO + PRX 10 mg/kg b.w.	1–2	1–3
mean RR [ms]	171.80 ± 11.87	160.00 ± 8.53	172.05 ± 6.99	p = 0.01	NS
max RR [ms]	188.32 ± 1.61	178.86 ± 10.79	188.74 ± 1.25	NS	NS
min RR [ms]	149.32 ± 18.94	145.45 ± 4.22	145.75 ± 12.57	NS	NS
range [ms]	37.10 ± 15.90	33.40 ± 12.94	42.97 ± 12.47	NS	NS
average HR [bpm]	351.00 ± 25.31	375.84 ± 18.75	349.18 ± 13.99	p = 0.01	NS
SDNN	7.83 ± 3.95	5.17 ± 3.20	12.52 ± 3.94	NS	p = 0.03
rMSSD	7.87 ± 8.34	3.00 ± 4.37	13.31 ± 12.15	NS	NS

NS = not significant

Table 2. Spectral-domain HRV analysis results in rats with experimental bladder outlet obstruction (BOO) model treated with piroxicam (PRX).

HRV spectral-domain parameter	Studied groups			Statistical analysis (for ln values)	
	group 1 BOO control	group 2 BOO + PRX 2 mg/kg b.w.	group 3 BOO + PRX 10 mg/kg b.w.	1–2	1–3
TP [ms^2]	27.44 ± 33.55	11.23 ± 20.45	18.23 ± 17.84	p = 0.04	NS
VLF [ms^2]	22.30 ± 28.95	6.61 ± 15.26	17.42 ± 17.47	p = 0.01	NS
LF [ms^2]	2.20 ± 3.26	2.32 ± 3.71	0.52 ± 0.42	NS	NS
HF [ms^2]	2.94 ± 6.27	2.30 ± 2.53	0.30 ± 0.26	NS	NS
LF/HF	0.71 ± 0.58	0.75 ± 0.70	1.85 ± 0.17	NS	p = 0.01
nLF [n.u.]	44.81 ± 18.59	35.25 ± 23.27	64.75 ± 2.12	p = 0.01	p = 0.01
nHF [n.u.]	55.19 ± 18.59	64.75 ± 23.27	35.23 ± 2.12	p = 0.01	p = 0.01

NS = not significant

Table 3. Time-domain HRV analysis results in rats with experimental hemorrhagic cystitis evoked by cyclophosphamide (CP-HC) model treated with piroxicam (PRX).

HRV time-domain parameter	Studied groups			Statistical analysis (for ln values)	
	group 4 CP-HC control	group 5 CP-HC + PRX 2 mg/kg b.w.	group 6 CP-HC + PRX 10 mg/kg b.w.	4–5	4–6
mean RR [ms]	160.17 ± 11.23	168.80 ± 14.66	172.77 ± 12.77	NS	NS
max RR [ms]	184.92 ± 5.75	188.95 ± 10.08	188.94 ± 10.06	NS	NS
min RR [ms]	146.57 ± 8.62	153.63 ± 20.01	147.49 ± 16.40	NS	NS
range [ms]	38.36 ± 13.81	35.32 ± 19.97	41.47 ± 16.38	NS	NS
average HR [bpm]	376.77 ± 26.16	357.58 ± 30.70	347.90 ± 24.40	NS	p = 0.04
SDNN	6.74 ± 2.62	8.80 ± 5.57	10.46 ± 5.17	NS	NS
rMSSD	5.48 ± 4.27	14.65 ± 12.97	15.08 ± 10.44	NS	p = 0.05

NS = not significant

Table 4. Spectral-domain HRV analysis results in rats with experimental hemorrhagic cystitis evoked by cyclophosphamide (CP-HC) model treated with piroxicam (PRX).

HRV spectral-domain parameter	Studied groups			Statistical analysis (for ln values)	
	group 4 CP-HC control	group 5 CP-HC + PRX 2 mg/kg b.w.	group 6 CP-HC + PRX 10 mg/kg b.w.	4–5	4–6
TP [ms²]	15.31 ± 15.22	46.77 ± 38.50	13.94 ± 9.49	NS	NS
VLF [ms²]	10.00 ± 9.35	27.8 ± 22.01	10.43 ± 6.85	NS	NS
LF [ms²]	2.01 ± 2.33	9.37 ± 8.01	2.07 ± 1.81	p = 0.05	NS
HF [ms²]	3.29 ± 3.83	9.61 ± 9.06	1.45 ± 0.98	NS	NS
LF/HF	0.71 ± 0.58	1.74 ± 1.35	1.34 ± 0.39	NS	NS
nLF [n.u.]	34.42 ± 18.77	57.86 ± 14.86	56.34 ± 6.81	p = 0.04	p = 0.04
nHF [n.u.]	65.52 ± 18.75	42.14 ± 14.86	43.66 ± 6.81	p = 0.04	p = 0.02

NS = not significant

HRV analysis in the CP-HC model

Time-domain analysis

Just like in case of the BOO model, parameters of the HRV time analysis in animals with the CP-HC model were not significantly different in both groups treated with PRX and in the control group 2. All values were comparable in all groups, except for a mean heart rate in the group 6 – the lowest of all groups, and rMSSD – the highest.

Results of the HRV time-domain analysis in animals in the CP-HC model are presented in Table 3.

Spectral-domain analysis

Contrary to animals in the BOO model (groups 1–3), in animals in the CP-HC model treated with the lower PRX dose (group 5) a trend for increase of all non-normalized components of the spectrum was observed, compared to the corresponding control group. In the group 6 (the higher PRX dose), similarly to groups 2 and 3, TP and HF values were lower. VLF and LF powers were practically identical as in the corresponding control group. Both groups 5 and 6 were characterized by dominance of the normalized nLF parameter over nHF, and the difference was statistically significant.

Results of the HRV spectral analysis of animals in the CP-HC model are presented in Table 4.

DISCUSSION

Considering literature reports, results of daily urinary output analysis, BWW values and histological valuation of collected bladders, a conclusion could be made that study animals (groups 1 and 4) meet requirements of bladder outlet obstruction (the BOO model) and of hemorrhagic cystitis (the CP-HC model). Hence, we could treat those groups and their HRV results as control ones, taking them as a reference for BOO/CP-HC individuals treated with piroxicam.

The most important conclusions of this study are:

1. Prostaglandins seem to be factors activating the autonomic nervous system in the model of bladder outlet obstruction – inhibition of their synthesis caused a reduction of power of the HRV spectrum and of its individual non-normalized components: VLF, LF and HF. The dose of 2 mg/kg b.w. caused a particular trend for reduction of power in the VLF range, with marked increase of value of the normalized nHF parameter; the 10 mg/kg b.w. dose caused mostly a reduction of LF and HF power, but accompanied by an increase of the normalized nLF value.

2. In the CP-HC model, the dose of piroxicam of 2 mg/kg b.w. caused an increase of global activity of the autonomic nervous system and its non-normalized components. That suggests that a moderate suppression of prostaglandin synthesis was reflected by increased activity of the ANS. At the higher PRX dose the stimulating effect on the ANS functional condition disappeared, and activity of the ANS was comparable to the corresponding control group. At both PRX doses a statistically significant dominance of the normalized nLF parameter was observed.

We have chosen piroxicam, as an agent inhibiting synthesis of prostaglandins and a non-selective COX inhibitor for our experiment. Piroxicam (4-hydroxy-2-methyl-2H-1,2-benzothiazine-1-(N-(2-pyridinyl)carboxamide)-1,1-dioxide) is a precursor of the oxicam subgroup in the group of NSAIDs. The drug was discovered in 1972 and introduced to

the medical market by Pfizer in 1982 as a novel – for that time – representative of NSAIDs, recommended particularly to pharmacotherapy of rheumatic problems (35). Piroxicam is characterized by high (about 30) COX-1/COX-2 blockade ratio value. Thus, this compound, together with others commonly used in clinical practice drugs, such as ibuprofen, ketoprofen, diclofenac, naproxen, belongs to NSAIDs that inhibit both COX-1 and COX-2 with little selectivity (36). However, comparing to the other non-selective NSAIDs mentioned above, piroxicam seems to be relatively the most non-selective COX-blocking agent. Thus, the pharmacodynamic profile of piroxicam was the reason of our choice of PRX in our experiment. Moreover, early other reports of additional pharmacodynamic properties of prioxicam appeared as well and other aspects of the agents' mechanism of action have been also discovered. Already in 1983, Ando and Lombardino (37) demonstrated that PRX inhibited cellular migration in an inflammatory focus, and stabilized lisosomes of neutrophils, thus counteracting release of numerous cellular pro-inflammatory mediators (and of the lymphocytic rheumatoid factor of the IgM antibody character). Summing up, we have chosen PRX for the puropose of our study due to its non-selectivity regarding to COX and potential additional antiinflammatory effects, even despite the fact, that this agent is nowadays rarely used in clinical practice (mostly because its gross COX non-selectivity produces serious adverse effect, such as unfavorable influence on the digestive tract). Compared to other NSAIDs, this agent also exerts a relatively strong ulcerogenic effect (35, 38). That fact was an impulse for search of new derivatives of the compound, concluded with discovery of PRX analogues, including meloxicam, tenoxicam and lornoxicam, differing from their predecessor not only in scope of pharmacodynamic properties (clearly higher selectivity in relation to COX-2), but also of pharmacokinetic properties (39).

As mentioned previously in the introduction, PGs locally synthesized in the bladder intensify its overreactivity. Therefore, it is expected that NSAIDs constitute a pharmacologically attractive group of agents exerting a potentially beneficial alleviating effect on OAB symptoms. Efficacy of selected NSAIDs has been confirmed in experimental studies, and in few – so far – published clinical trials. In an experimental model of hemorrhagic cystitis induced by a single, large dose of cyclophosphamide (150 mg/kg b.w.), Takagi-Matsumoto et al. (40) demonstrated an improvement of parameters describing cystometric records in rats, in which the records were registered during an intravesical administration of a selected non-selective COX inhibitor. Those researchers evaluated an effect of aspirin (0.1–10 mg/kg b.w.), indometacin (0.01–0.3 mg/kg b.w.) and ketoprofen (0.001–0.1 mg/kg b.w.). Results obtained by them confirmed efficacy of each of the tested agents in scope of alleviation of cystometric disorders determining OAB in studied individuals (they observed a reduction of frequency of micturition episodes and a shift towards the higher value of threshold micturitional pressure during the phase of bladder filling). A significant improvement of cystometric properties of those records was accompanied by reduction of intravesical PGS level, evaluated immunoenzymatically in the vesical supernatant (40). Results of the above experiment were also confirmed by Jang et al. (41) on a similar experimental model of OAB, but with use of a selective COX-2 inhibitor (rofecoxib). Moreover, those researchers evaluated the nerve growth factor (NGF) and expression of the induced isoform of nitrogen oxide synthase (iNOS) content in vesical tissues. They demonstrated that rats with cyclophosphamide-induced chemical bladder injury receiving rofecoxib at the dose of 2 mg/kg b.w. for one hour, during the urodynamic record, were characterized by significantly lower content of both NGF and iNOS. In opinion of those authors, that results also in reduced COX-2 expression in bladders of experimental animals when considering physiological premises: NGF induces activity of COX-2 and production of PGs by vesical mastocytes, and increased enzymatic activity of iNOS and COX-2 depends on the presence of a common activator of both enzymes (nitrogen peroxide, formed in the course of NO metabolism in a focus of inflammation) (41). The theory of improvement of bladder disturbances as a result of reduced intravesical PGs synthesis by COX inhibition has been supported also by Shioyama et al. (42). Those researchers, using a different OAB model (chemical damage of the urothelium induced by intravesical administration of protamine sulfate), demonstrated that administration of loxoprofen for two weeks alleviated symptoms of bladder overreactivity.

There are also few – so far – published clinical reports on favorable effect of NSAIDs in OAB pharmacotherapy. As mentioned before in the introduction, in the BOO model, reflecting BPH, an increase of the intravesical PGs level was observed. Saito et al. (43) demonstrated a significant reduction of symptoms of bladder overactivity during night in BPH patients treated with loxoprofen (the drug caused reduction of nycturia episodes). Similarly,

Ozdemir et al. (44), evaluating BPH patients treated with the combined therapy doxazosin and tenoxicam, with use of validated questionnaire scales IPSS (International Prostate Symptom Scale) and OABSS (Overactive Bladder Symptom Scale), demonstrated improvement of their quality of life compared to the monotherapy with doxazosin.

Summing up, the review of literature confirms that inhibition of intravesical PGs synthesis with NSAIDs is associated with alleviation of overactive bladder symptoms. In our opinion, that favorable effect may be also – at least partially – associated with a change of autonomic regulation of the bladder, resulting from disappearance of the modulating role of prostaglandins. That hypothesis is supported by our HRV analysis results, particularly the spectral one (time-domain analysis revealed practically no statistically significant differences between groups).

As mentioned above, in the BOO model (corresponding to BPH), using both piroxicam doses, we found a reduction of values of non-normalized spectrum components (HF, LF) and of the total power (TP) of HRV. Additionally, the low PRX dose was associated with increased power of the normalized component nHF and a significant reduction of the VLF value, and the large one – with an increase of the normalized component nLF. On the other hand, in CP-HC animals (the model clinically corresponding to hemorrhagic, post-cyclophosphamide bladder injury), depending on the dosage, we demonstrated an increase of the spectrum total power and of its individual non-normalized components (2 mg/kg b.w. PRX), or – just like in BOO – their reduction). However, regardless the applied PRX dose, in that model we observed a statistically significant dominance of the normalized parameter nLF. According to HRV interpretation guidelines (26, 27), sympathetic activity is expressed mostly by nLF, parasympathetic – reflected by HF and nHF powers, and the TP value is associated with the global ANS tonus. There is no general consensus regarding an unequivocal interpretation of the LF component – a majority of researchers perceive that parameter as an expression of activity of both arms of the autonomic system. Even greater controversies are associated with the VLF component, which – in opinion of majority – could be an expression of various processes associated with hemodynamic regulation dependent on the sympathetic control (26, 27, 45). There is also evidence supporting the hypothesis that VLF reflects the activity of cholinergic anti-inflammatory pathway, and hence, the component is of parasympathetic origin (46, 47).

Considering the above guidelines, results obtained by us from the HRV analysis in the BOO model suggest that PGs seem to be agents stimulating global activity of the ANS, since inhibition of their synthesis causes reduction of the HRV spectrum. Evaluating non-normalized spectrum components only, it seems that the global reduction is accompanied by a proportional reduction of activity of both ANS arms. However, that thesis is negated by values of normalized parameters nLF and nHF. In our opinion, lower COX block is expressed mainly in reduced sympathetic tonus, as evidenced by reduction of nLF and VLF (accepting the hypothesis of a dominating role of the sympathetic component in generation of power of that component). COX blockage achieved with the 2 mg/kg b.w. dose and the functional sympathetic withdrawal contributed to an increased parasympathetic tonus (nHF increase), that may still fix symptoms of OAB (high parasympathetic activity stimulates contractile activity of the bladder (5)). Five times higher PRX dose (10 mg/kg b.w.; group 3), with reduction of the total vegetative reactivity, caused also reorganization of the balance within the scope of two essential components of ANS, with a sympathetic dominance described by increased nLF, that accounts for reduction of symptoms of bladder overactivity. High sympathetic activity, associated with activation of β-adrenergic receptors in the bladder, leads to a decrease of its contractile activity (5). Therefore, administration of 10 mg/kg b.w. PRX and inhibition of PGs synthesis causes changes of autonomic regulation consistent with the above cited reports regarding the alleviating effect of NSAIDs on symptoms of OAB.

Similar conclusions as those regarding group 3 may be drawn analyzing results of the spectral analysis of animals in the CP-HC model, that received PRX at the dose of 10 mg/kg b.w. (group 6). Also in that population, obtained results suggest reduction of global activity of the ANS, with a vegetative balance shifted towards the sympathetic component (reduced TP, HF, nHF values with increased nLF). However, results obtained in the group of animals with the CP-HC model but treated with a lower PRX dose (2 mg/kg b.w.; group 5) are debatable. That was the only subpopulation for which we observed a paradoxical increase of TP (increase of the ANS total activity), and of both non-normalized spectral components LF and HF (suggesting increase of both sympathetic and parasympathetic tonus). However, even in that group we observed identical relations regarding normalized parameters nLF and nHF, with a dominance of nLF, suggesting an increased sympa-

thetic tonus with COX block. We found interpretation of the fact of increased TP and all non-normalized components : VLF, LF and HF in that model and at that PRX dose, difficult. Undoubtedly, in that model, post-cyclophosphamide inflammatory changes were clearly more intense, compared to BOO. A lower PRX dose, despite its inhibitory effect on COX and PGs synthesis, probably was insufficient to reduce the activating effect of numerous other pro-inflammatory mediators on the ANS. Moreover, with increasing PRX dose, an additional, inhibitory aspect of PRX effect on activity of immunocompetent cells, as mentioned above, could appear (37). Therefore, only the higher PRX dose caused changes similar to those observed in the BOO model, with establishment of identical relations between the sympathetic and parasympathetic activity.

CONCLUSIONS

Summing up, results of our experiment support the potential efficacy of NSAIDs as PGs synthesis inhibitors in reduction of bladder overactivity in both experimental models of OAB (chemical – CP-HC and obstructive – BOO), reported in the literature. In the BOO model, PGs seem to be ANS-activating factors, and in the CP-HC model – they are undoubtedly co-activators together with other proinflammatory mediators. The dose of 10 mg/kg b.w. of piroxicam in both models caused a reconstruction of the autonomic balance, with marked sympathetic dominance, and with simultaneous reduction of total tonus of the ANS. That suggests that PGs inhibit the sympathetic activity, and directly or indirectly intensify the parasympathetic activity, contributing to contractile overactivity of the bladder. Functional rearrangement of the ANS following inhibition of PGs synthesis may cause an opposite phenomenon (reduction of contractile activity of the bladder), which undoubtedly improves bladder compliance during its filling.

Of course, we are aware of limitations of our study (no biochemical and histological studies were made, that could objectively confirm inhibition of prostaglandin synthesis and changes in ANS activity). Therefore, our results have to be treated as preliminary and requiring further clarification. Moreover, the applied high PRX dose (10 mg/kg b.w.), exceeding the usually clinical applied dose, certainly would be a causative factor of severe gastric ulceration and renal damage in humans. However, in our opinion, supporting the general concept of beneficial effect of NSAIDs on disorders associated with OAB, they are valuable, as they indicate some changes in autonomic regulation as a possible mechanism of NSAIDs action in OAB. Moreover, the additional actions of NSAIDs revealed in our study also provide new insights into pharmacodynamics aspects of these agents.

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